

AD\_\_\_\_\_

Award Number: DAMD17-01-1-0729

TITLE: Molecular Epidemiology of Ovarian Cancer

PRINCIPAL INVESTIGATOR: David Bowtell, Ph.D., D.V.M./V.M.D.  
Adele Green, Ph.D.  
Georgia Chenevix-Trench, Ph.D.  
Anna DeFazio, Ph.D.  
Dorota Gertig, Sc.D.  
David Purdie, Ph.D.  
Penelope Webb, Ph.D.  
David Whiteman, Ph.D.

CONTRACTING ORGANIZATION: University of Melbourne  
East Melbourne, Victoria 3002  
Australia

REPORT DATE: September 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

<b>1. AGENCY USE ONLY</b> (Leave blank)		<b>2. REPORT DATE</b> September 2004	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (15 Aug 2003 - 14 Aug 2004)	
<b>4. TITLE AND SUBTITLE</b> Molecular Epidemiology of Ovarian Cancer			<b>5. FUNDING NUMBERS</b> DAMD17-01-1-0729	
<b>6. AUTHOR(S)</b> David Bowtell, Ph.D., D.V.M./V.M.D., Adele Green, Ph.D., Georgia Chenevix-Trench, Ph.D., Anna DeFazio, Ph.D., Dorota Gertig, Sc.D., David Purdie, Ph.D., Penelope Webb, Ph.D., David Whiteman, Ph.D.			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> University of Melbourne East Melbourne, Victoria 3002 Australia  <i>E-Mail:</i> d.bowtell@petermac.org				
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b> Original contains color plates: All DTIC reproductions will be in black and white.				
<b>12a. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited				<b>12b. DISTRIBUTION CODE</b>
<b>13. ABSTRACT (Maximum 200 Words)</b> <p>The aim of this Program is to study the association between epidemiologic risk factors, low-risk genes, and histologic and novel molecular subtypes of ovarian cancer. In December 2002, we received final approval from the Human Subject Research Review Board (HSRRB) to start recruitment at 13 of the proposed study sites and have since received approval to recruit at a further 2 sites. We have established a network of research nurses across the country and recruitment is now progressing well at 15 different sites. Recruitment of cases for the study began in January 2003 and control recruitment began in May 2003 and we have since recruited a total of 934 women with ovarian cancer and 569 control women. The recruitment, sample and data collection and processing systems are working well and we are continuously monitoring our performance against our targets as outlined in the reports from Core Components A (Epidemiology) and B (Biospecimens). In relation to this we also have established systems to manage data and samples from all of the different sites.</p>				
<b>14. SUBJECT TERMS</b> Molecular epidemiology, heterogeneity, histologic subtypes, ovarian cancer				<b>15. NUMBER OF PAGES</b> 16
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b> Unlimited	



## TABLE OF CONTENTS

---

Cover Page .....	1
Standard Form 298 .....	2
Table of Contents .....	3
Introduction .....	4
Body .....	4
Key Research Accomplishments .....	15
Reportable Outcomes .....	15
Conclusions .....	15
References .....	16
Appendices .....	16

---

## ANNUAL REPORT

Title: Molecular Epidemiology of Ovarian Cancer: The Australian Ovarian Cancer Study,"  
 Proposal No. OC000109  
 Award No. DAMD17-01-1-0729  
 HSRRB Log No. A-10884.

## INTRODUCTION

The aim of this Program is to study the association between epidemiologic risk factors, low-risk genes, and histologic and novel molecular subtypes of ovarian cancer, thereby addressing the heterogeneity of the disease and of susceptibility to environmental exposures. To this end, we have established a multi-center population-based resource involving collection of linked epidemiologic and clinical data and biospecimens from cases and matched controls.

## BODY

## Cores A and B

In December 2002, we received final approval from the Human Subject Research Review Board (HSRRB) to start recruitment at 13 of the proposed study sites. During the final months of 2002 we identified Research Nurses for each study site and set up procedures at each hospital. By January 2003 research nurses were deployed at the collecting sites and we started phasing in recruitment. We now have HSRRB approval to recruit at all 16 sites listed below.

Recruitment and data/sample collection is proceeding well (see Reports for Cores A, Epidemiology, and B, Biospecimens, below).

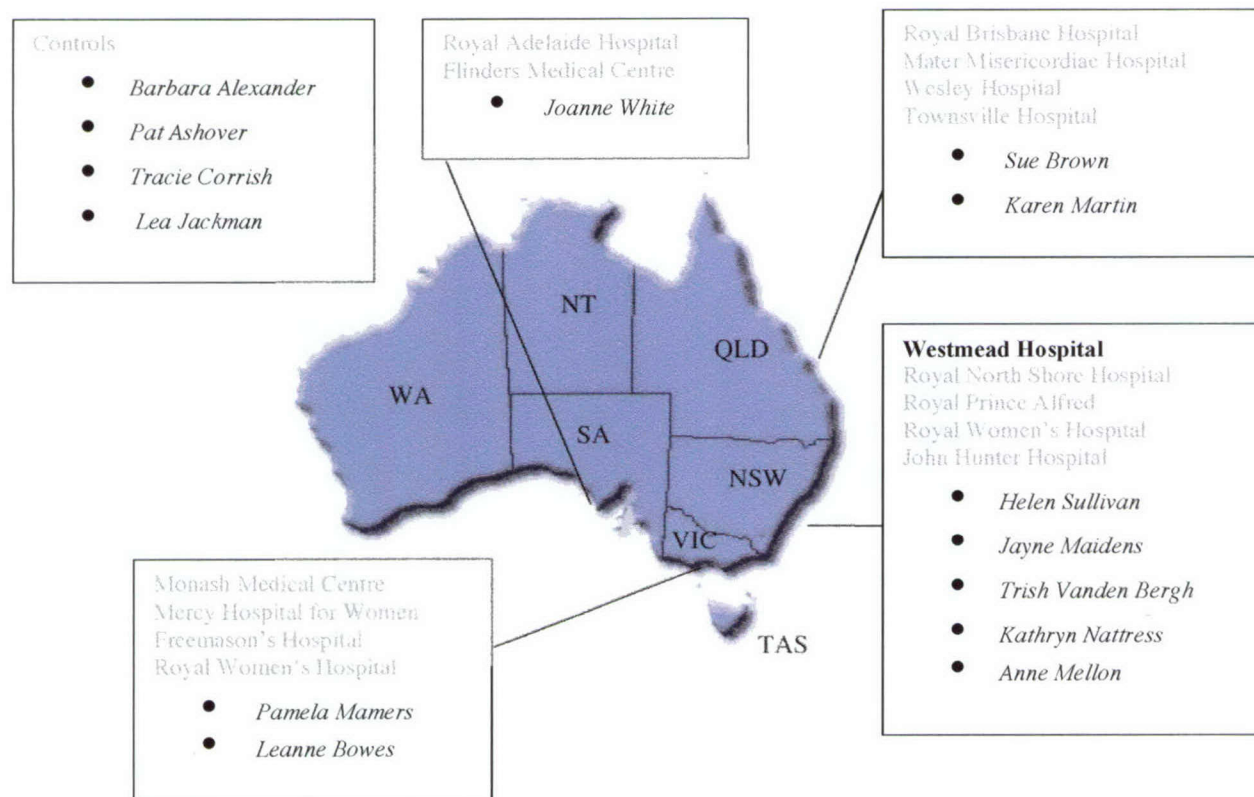


Figure 1: Australian Ovarian Cancer Study Site and RN network at 14 September 2004

## **Tasks Outlined in the Approved Statement of Work**

### **Core A: Epidemiology**

#### **Task 1- Preliminary Work (Prior to start date)**

- (a) **Data-collection instruments will be finalised and piloted based on practical experience in a previous study (Survey of Women's Health)**

Completed – see 2002 Annual Report.

- (b) **Institutional Review Board approval will be obtained from all participating hospitals and institutions (to be prior to start date)**

Completed – see 2003 Annual Report.

- (c) **Identification of project manager, data manager and nurse-interviewers to start on Day 1.**

Completed – see 2003 Annual Report.

#### **Task 2- Set-up (months 1-2)**

- (a) **Finalise details of case identification system in each of the major centres (month 1)**

Completed – see 2003 Annual Report.

- (b) **Training of interviewers in Brisbane (month 1)**

Completed – see 2003 Annual Report.

- (c) **Development of computer data-bases (Access) for data-entry  
Recruitment "Tracking" Database**

Completed – see 2003 Annual Report.

#### **Main Questionnaire and Dietary Questionnaire Databases**

We have developed and tested the database for the main study questionnaire, the dietary questionnaire and the interview and data entry is on-going (see below). The databases incorporate multiple range and logic checks to prevent errors in data entry.

#### **Task 3- Recruitment of cases (n>1000). (Ongoing months 2-36)**

- (a) **Cases will be identified by the nurse-interviewers on an ongoing basis through participating hospitals and clinics with additional checks run through the state cancer registries**
- (b) **Treating physicians will be contacted to obtain permission to contact the case**
- (c) **Cases will be contacted and interviewed and biological samples collected**
- (d) **Tumor blocks and copies of pathology records will be obtained**

Recruitment was phased in from 1 January 2003 and is going well - see summary table below.



**Case ascertainment:** The total numbers of eligible cases in each state are estimated from numbers of ovarian cancers registered in each state in 2000 (the most recent data available). We were aiming to ascertain 100% of cases in Qld and SA and 50% in Victoria and New South Wales and have met these targets in NSW, Qld and SA. Recruitment at one major hospital in Victoria did not start until 1 Jan 2004 hence ascertainment in Vic is currently slightly below the target of 50% but we anticipate this figure will increase now we are recruiting at all the major sites there.

**Response rate:** We were aiming for a response rate of 85-90% and, to date, 74% of cases have consented currently. This is a significant underestimate of the final consent rate as some cases have been identified but have not yet had time to return their consent forms. The patient refusal rate is currently 11% overall suggesting the final participation rate will be between 85 and 90%. We are well on track to achieve our target of 1000+ cases.

**Data and sample collection:** Questionnaire return and blood, tissue and urine collection lag slightly behind consent thus these figures are under-estimates of final completion rates. Sample collection will be discussed further under the Biospecimen Core Report.

#### Case Recruitment 1 Jan 2003 – 31 August 2004

	NSW	QLD	SA	VIC	TOTAL
<b>Total cases (estimated)<sup>1</sup></b>	<b>550</b>	<b>271</b>	<b>141</b>	<b>550</b>	
<b>Cases ascertained</b>	<b>349</b>	<b>376</b>	<b>138</b>	<b>190</b>	<b>1053</b>
% of total (see Fig 2)	63%	139%	98%	35%	
<b>Cases included<sup>2</sup></b>	<b>317</b>	<b>320</b>	<b>118</b>	<b>179</b>	<b>934</b>
% of those ascertained	91%	85%	86%	94%	89%
<b>Consents<sup>3</sup></b>	<b>228</b>	<b>217</b>	<b>92</b>	<b>156</b>	<b>693</b>
% of included	72%	68%	78%	87%	74%
<b>Q returned to QIMR<sup>3</sup></b>	<b>133</b>	<b>167</b>	<b>60</b>	<b>94</b>	<b>454</b>
% of consents	58%	77%	65%	60%	66%
<b>Fresh tissues<sup>3,4</sup></b>	<b>141</b>	<b>96</b>	<b>49</b>	<b>96</b>	<b>382</b>
% of consents	62%	44%	53%	62%	55%
<b>Blood samples<sup>3</sup></b>	<b>176</b>	<b>196</b>	<b>62</b>	<b>132</b>	<b>566</b>
% of consents	77%	90%	67%	85%	82%
<b>Urine samples<sup>3</sup></b>	<b>161</b>	<b>101</b>	<b>0</b>	<b>70</b>	<b>332</b>
% of consents	71%	47%	0%	45%	48%

Table 1. Summary collection of cases and biospecimens

<sup>1</sup> Based on number of cases diagnosed in 2000 (most recent national data available), assuming we identify 50% of cases in NSW and Vic

<sup>2</sup> Women were excluded if they were not able to give informed consent (eg non-English speaking, psychological reasons, too sick)

<sup>3</sup> Questionnaire completion (and sometimes sample collection) occur some weeks after recruitment and surgery thus these numbers lag behind the number consented and do not represent final figures.

<sup>4</sup> Our target was to collect 600 fresh tissue samples. Early collection was slow in some centres but procedural problems encountered initially have largely been addressed and we anticipate achieving this total.

#### Task 4- Recruitment of population controls (n>1000). (Ongoing months 2-36)

- (a) **Potential controls will be selected at random through the Commonwealth electoral roll on a weekly basis and frequency matched by age and geographic region to the distribution of cases identified the previous week**
- (b) **Invitation letters will be sent to controls**
- (c) **Telephone follow-up of controls**
- (d) **Interview of controls and collection of blood and urine samples**

We began control recruitment in late March 2003. To date 569 women have consented or provisionally agreed to take part. *[Note: this represents 44% of those initially approached but does not allow for women who have been approached but have not yet responded and some ineligible women have to be excluded from the denominator. The final response rate will thus be considerably higher than 44%].* We are on track to reach our target of 1000 control women by mid-late 2005. The questionnaire return rate and blood collection rate are currently greater than 85%. These are also not final figures because we do not yet have questionnaires/samples for women who only recently consented. The final return rates will thus be higher than this.

#### **Control Recruitment 1 April 2003 – 14 September 2004**

	<b>TOTAL</b>
<b>Consents</b>	<b>437</b>
<b>Q returned</b> % of consents <sup>1</sup>	<b>390</b> 89%
<b>Blood samples</b> % of consents <sup>1</sup>	<b>375</b> 86%

<sup>1</sup> Questionnaires are returned and blood collected some time after consent is obtained thus these numbers do not represent final figures.

<sup>2</sup> We are still setting up procedures for urine collection from control

#### **Task 5- Data entry / checking / cleaning. (ongoing months 3-42)**

- (a) **Data will be entered into the databases on an ongoing basis**
- (b) **Data will be cleaned using frequency and range checks, implausible values will be cross-checked against the original questionnaires and corrected if necessary**

We have started data entry for the study questionnaires. We are currently running logic and range checks for the data entered and have started data cleaning.

#### **Task 6- Data management (ongoing months 37-48)**

We are establishing algorithms to create derived variables for future analyses. This will enable us to commence full data-analysis in a timely fashion after data entry is complete.

#### **Core B- Biospecimens**

##### **Task 1- Preliminary Work (Prior to start date)**

- a) **Institutional Review Board approval will be obtained from all participating hospitals and institutions (to be prior to start date)**



Completed – see 2003 Annual Report.

**b) Recruitment of data manager and specimen processing staff**

Completed – see 2003 Annual Report.

**c) Further refinement of computer data-bases (Access) for data-entry**

Completed- See 2002 Annual Report.

**Task 2- Set-up (months 1-2)**

**a) Finalise details of case ascertainment system in each of the major centres (month 1)**

Completed- See 2002 Annual Report.

**b) Obtain minor equipment and consumables**

Completed- See 2002 Annual Report.

**Task 3- Ascertainment of samples. (Ongoing months 2-36)**

- a) Nurse-interviewers to liaise with Biorepository head, notifying of incoming shipment of samples**
- b) Nurse-interviewers to provide Biorepository staff with details of pathology blocks from cases to be requested. Biorepository staff to coordinate temporary block acquisition**
- c) Blood and urine samples shipped overnight from national sites. Fresh frozen samples from interstate stored at centres at  $-80^{\circ}\text{C}$ , shipped on dry ice at monthly intervals. Blocks from pathology clinics requested on a monthly basis.**

As discussed above, a team of part-time nurse interviewers are in place at hospitals across Australia and sample collection for the biospecimen core has been in effect since January 1 2003.

Sample collection is going well. Blood samples are shipped at room temperature on a daily basis to the Biorepository and nurse-interviewers liaise directly with the Biorepository head, notifying of incoming samples. This is usually via email. Fresh frozen tissue samples and urine samples are shipped on dry ice on a monthly basis via overnight courier. Again, the nurse-interviewers notify the Biorepository head of all incoming samples.

Nurse-interviewers collect both fresh frozen and fixed tissue samples. The fixed samples shipped to PMCC on a daily basis and are processed into blocks. This means that it is no longer necessary for the biorepository staff to co-ordinate temporary access to the diagnostic blocks to obtain a slide.

In addition, an expert gynaecological pathology review panel has been established to review all cases recruited to the study. On a monthly basis, the diagnostic slides from pathology centres (where the case was first diagnosed) are called in for centralized



review. This protocol has been in place for several years to facilitate ovarian cancer research in Australia and is being co-ordinated by Dr Peter Russell and the Biorepository head.

Table 1 shows the number of tissue, blood and urine samples collected since January 1 2003. We anticipate collection of 600 fresh tissue samples over the 3 years of the study (60%). Currently, tissue collection rates stand at 55%,

#### **Task 4. Sample processing and dispatch. (Ongoing months 2-36)**

- a) **Incoming samples of blood, urine, fresh frozen tissue and blocks to be processed as described in methods**
- b) **Requested samples shipped to centres in general on a monthly basis but immediately available if needed**
- c) **Sample backup to QIMR sent as batches on a monthly basis**
- d) **Periodic quality control procedures to validate sample integrity**

Joy Hendley and Lisa DiPrinzio are responsible for all biospecimen sample processing. Incoming blood, urine and tissue (frozen and fixed/blocks) samples are processed according to the AOCS protocols. A backup sample is stored separately (liquid nitrogen and -80°C) and sent via overnight courier to QIMR on a monthly basis.

We have established quality control protocols and the biorepository staff are responsible for their implementation.

#### **Task 5. Data entry / checking / cleaning. (ongoing months 3-42)**

- a) **Data will be entered into the databases on an ongoing basis**
- b) **Provide data for analysis as required**

Biorepository staff are responsible for entering information regarding sample collection (type of sample, date collected, date processed) and processing (fractions processed, amount processed, storage location) onto the biospecimen database.

The Data Manager, Sian Fereday, is responsible for generating monthly statistics for the management group meetings. These reports describe primary site, histology subtype and stage of the biospecimens collected.

Adhoc data requests are furnished as required.

---

### **Project 1: Molecular Subtype Analysis of Ovarian Cancer**

#### **Task 1. Initial DNA microarray analysis with ~300 archival fresh frozen samples (months 1-12)**

Utilising retrospectively collected specimens of Ovarian Cancer (OvCa) a database of gene expression information has been created. The data were generated using custom printed cDNA microarray slides from The Peter MacCallum Cancer Centre Microarray Facility. These arrays contain approximately 10,500 genetic elements and have been used to profile differences in gene expression between various clinically important classes of OvCa. Over 100 samples have been profiled to date and the data analysed to investigate



three major questions: correlation of expression profile with outcome, classification of Low Malignant Potential (LMP) tumours, and classification of Krukenberg tumours.

**Outcome:** Genes with robustly different patterns of expression between patients with short or long survival times may be important targets for novel diagnostic or therapeutic tools. To explore this potential, analysis of array data generated from patients following standard treatment regimes was conducted. Groups of molecular profiles from patients with survival times <12 months, 12-24 months and >24 months were created. Pattern recognition algorithms were used to identify genes from the 10,500 available, whose expression correlated significantly with the survival phenotype. From this analysis a prediction model was created which was able to correctly assign patients to one of either three survival categories with approximately 80-90% accuracy. In addition, a linear formula was generated, which was capable of predicting survival time as a continuous variable from gene expression data. Importantly genes identified by the algorithms used in this study included several genes of known importance in OvCa prognosis, including TYROBP, CXCL9, CCL8 and MT1G as well as several other genes not previously shown to correlate with patient outcome. Kaplan Meier analysis of the predictions made from this analysis revealed a highly statistically significant difference between survival classes. Further investigation and validation of these findings is planned using additional specimens from Westmead hospital (see Task 2 below)

**LMP tumours:** LMP tumours are an unusual class of ovarian tumours that display a relatively indolent pattern of disease, despite frequently having mutations in the ras-MAPK pathway. We have used microarrays to explore global gene expression patterns differences in mucinous invasive, mucinous LMP, serous invasive and serous LMP tumours. A multivariate method of microarray data analysis has been conducted whereby variation in expression attributable to histology, for example, is controlled for thus revealing genes with expression patterns influenced by or responsible for the phenotypes of interest. Gene ontology, genomic and pathway information was used to elucidate the molecular processes that differentiate LMP from invasive tumours and we have compared these with similar datasets for other solid cancers, particularly breast cancer. In addition, we are comparing expression patterns of LMP tumours for which we have determined their k-ras and BRAF mutation status.

We have identified a set of genes with robust expression differences between LMP and invasive tumours. This subset contains a significant representation of antigen presentation, cell-cycle regulation, control of cell growth and adhesion genes. We have shown that the LMP/invasive expression signature is similar to that observed in published expression analyses of breast ductal carcinoma in-situ versus invasive ductal carcinoma, and low versus high-grade breast cancer.

A highly significant proportion of the genes that discriminate between LMP and invasive ovarian cancer were located on chromosome six ( $P < 0.001$ ). In particular, large differences in expression of genes at previously published areas of LOH on chromosome 6. Gene expression in these areas, known to contain tumour suppressor and apoptosis regulating genes, appears to be strongly inversely correlated between invasive and LMP tumours, with areas of under expression in invasive tumours associated with previously defined regions of LOH and gain, with regions of amplification. These findings suggest that copy number and gene expression changes on chromosome 6 may fundamental differences in growth and invasion between invasive and LMP tumours.



**Krukenberg tumours:** Krukenberg tumours are gastric tumours that metastasize to the ovary but other sites, including pancreas, breast and colon are common sites of origin for Krukenberg-like tumours. Whilst patients with these tumours may be recognized at surgery or upon pathological assessment, the clinico-pathological picture is often uncertain or such patients may simply go unnoticed. Treatment of such cases with a platinum-based regimen is usually ineffective. Our findings indicate that expression profiling allows the rapid identification of unrecognized metastases to the ovary and may be of use in the clinical management of the disease.

We have also developed a machine learning-based test to more accurately diagnose ovarian cancers. Initially, a cross-validated model of gene expression in primary ovarian cancer vs. over 100 other primary tumours was created and applied to LMP/invasive tumours to ensure the dataset is not contaminated by metastases from other tissues. AOCS has provided a very large, population-based, series in which we can estimate frequencies of primary and secondary mucinous cancers of the ovary using newer pathological criteria and our molecular classification. We have found that a higher fraction of invasive mucinous ovarian tumours represent secondaries, rather than primary tumours, than generally appreciated. We believe that the classifier may be used in conjunction with other clinical parameters to facilitate the diagnosis of mucinous invasive tumours of the ovary.

**Task 2. Progressively switch to microarray analysis of prospectively collected samples (months 12-42)**

The inclusion of prospective samples is commencing now. We have now switched further microarray expression analysis of ovarian cancer samples to an Affymetrix U1332.0+ array system. This has advantages of industrial level quality control, increased array complexity and particularly, will facilitate comparison of other studies with this very large dataset. The intention is to array 500 samples, including previously tested archival samples plus the, as yet untested, prospective samples on U1332.0+ arrays.

**Task 3. Ongoing statistical analysis of expression results (months 3-42)**

See Task 1 above.

**Task 4. Full statistical analysis of expression data and preparation of manuscripts (months 42-48)**

---

**Project 2: Determinants of Epithelial Ovarian Cancer- by histologic subtype and tumor behaviour**

This project will not formally commence until epidemiologic data collection is complete and it will run through the 4<sup>th</sup> year of the program. During Year 4 analysis of the specific hypotheses will proceed in parallel under the guidance of the PI and Co-investigators.

---

**Project 3: Low-risk genes for epithelial ovarian cancer**

**Task 1. To establish the 16 single nucleotide polymorphism (SNP) genotyping assays, including identification of genotyping controls (months 1-18)**

**Task 2 To genotype the cases from the Survey of Women's Health Study and controls from the Australian Breast Cancer Family Study for 16 SNPS (months 6-24)**

Genotyping has been completed for ~550 ovarian cancer cases and 300 healthy controls for 11 SNPs as indicated in the table below. Four SNPs have been excluded from genotyping because of their low frequency (0-0.5%) detected in a sample of 90-125 Australian controls [published frequencies: 4% for *HSD17B1*:A-27C (Peltoketo et al, 1994); 2.4% for *RAD50*:Arg884His (<http://greengenes/llnl.gov/dpublic/secure/reseq>) and 2% for *RAD52*:Ser347Ter (Han et al, 2002)].

Gene	Polymorphism	Status
<b>Androgen Receptor (AR)</b>	CAG <sub>n</sub>	Spurdle et al., 2002
<b>Progesterone Receptor (PR)</b>	C44T	Not Commenced
<b>Progesterone Receptor (PR)</b>	G331A	Berchuck et al., 2004
<b>Aromatase (CYP19)</b>	C>T 3'UTR	Completed
<b>5alpha-reductase (SRD5A2)</b>	Val89Leu	Completed
<b>17βhydroxysteroid dehydrogenase (HSD17B1)</b>	A-27C	Excluded
<b>17βhydroxysteroid dehydrogenase (HSD17B1)</b>	Ala238Val	Completed
<b>17βhydroxysteroid dehydrogenase (HSD17B1)</b>	Ser313Gly	Completed
<b>17βhydroxysteroid dehydrogenase (HSD17B4)</b>	Trp511Arg	Completed
<b>BRCA2</b>	Asn372His	Auranen et al., 2003
<b>X-ray cross complementation (XRCC2)</b>	Arg188His	Webb et al., 2004
<b>X-ray cross complementation (XRCC3)</b>	Thr241Met	Webb et al., 2004
<b>X-ray cross complementation (XRCC3)</b>	CA <sub>n</sub>	Not Commenced
<b>RAD50</b>	Arg884His	Excluded
<b>RAD52</b>	Ser347Ter	Excluded
<b>RAD52</b>	Tyr418Ter	Keleman et al, 2004

Analysis of the *RAD52* Y415Ter, *XRCC2* R188H G>A and *XRCC3* T241M C>T polymorphisms revealed no difference in genotype distribution between cases and controls. There was no increased risk of cancer associated with heterozygous genotype of *RAD52* Y415Ter (OR 0.55; 95% CI 0.24-1.24) (Keleman et al., 2004); *XRCC2* GA/AA genotype (OR 0.77; 95% CI 0.51-1.14), or with the *XRCC3* CT or TT genotypes (OR 0.80; 95% CI 0.59-1.09 and OR 0.92; 95% CI 0.58-1.44, respectively) (Webb et al., 2004). There was also no indication that genotype frequency differed across ovarian cancer subgroups defined by tumour characteristics, including histology. P53 positive tumours seemed to be over-represented in carriers of the *RAD52* truncation



polymorphism (100% of 7 carriers but only 64% of 142 non-carriers were p53 positive). Although the rarity of the variant genotype frequency provided little power to detect modest risks in cancer for the *RAD52*, *XRCC2* and *XRCC3* variants, the data suggest that none of these variants play a major role in predisposition to ovarian cancer risk at the population level.

In contrast, in collaboration with Drs Easton et al in Cambridge, we have found an association between the Asn372His genotype of *BRCA2* and ovarian cancer risk (Auranen et al., 2003). We genotyped a total sample of 1121 ovarian cancer cases and 2643 controls. There was no difference in genotype frequency between control groups from the two Australian and British studies ( $P=0.9$ ). The *HH* genotype was associated with an increased risk of ovarian cancer in both studies, and the risk estimate for the pooled studies was 1.36 (95% CI 1.04-1.77,  $P=0.03$ ). There was also a suggestion that this risk may be greater for ovarian cancers of the serous subtype for both studies, with an OR (95% CI) of 1.66 (1.17-2.54) for the two studies combined ( $P=0.005$ ). The *BRCA2* 372 *HH* genotype appears to be associated with an increased risk of ovarian cancer of a similar magnitude to that reported for breast cancer.

There was no evidence for a relationship between the variant allele and ovarian cancer risk for *CYP19* or *HSD17B1* [age adjusted OR (95%CI): *CYP19* 3'UTR heterozygous CT genotype 0.86 (0.60-1.24), *CYP19* 3'UTR homozygous TT genotype 0.70 (0.47-1.07); V allele of *HSD17B1* A238V 1.38 (0.35-5.49); heterozygous genotype of *HSD17B1* S313G 1.22 (0.87-1.72), homozygous GG genotype of *HSD17B1* S313G 0.98 (0.65-1.47)]. These odds ratios remained largely unchanged when tumours of low malignant potential (LMP) were excluded from the analysis. The genotyping protocol used for *HSD17B1* was used in the paper by Dunning et al (2004), on which Ms Livia Keleman (DoD-funded research assistant) is an author.

The +331G/A SNP in the progesterone receptor gene, *PR*, was genotyped in a population-based, case-control study from North Carolina (438 cases and 504 controls) and in 535 cases and 298 controls from the Survey of Woman's Health (Berchuck et al., 2004). This SNP, which is in the *PR* promoter, alters the relative abundance of the A and B isoforms and has been associated with an increased risk of endometrial and breast cancer. There was a suggestion of a protective effect of the +331A allele (AA or GA) against ovarian cancer in the North Carolina study (OR = 0.72, 95% CI = 0.47-1.10). Examination of genotype frequencies by histologic type revealed that this was due to a decreased risk of endometrioid and clear cell cancers (OR = 0.30, 95% CI = 0.09-0.97). Similarly, in the Australian study there was a non-significant decrease in risk of ovarian cancer among those with the +331A allele (OR 0.83, 95% CI = 0.51-1.35) that was strongest in the endometrioid/clear cell group (OR 0.60, 95% CI = 0.24-1.44). In the combined US/Australian data that included 174 endometrioid/clear cell cases (166 invasive, 8 borderline) the +331A allele was significantly associated with protection against this subset of ovarian cancers (OR 0.46, 95% CI = 0.23-0.92). Preliminary evidence of a protective effect of the +331A allele against endometriosis also was noted in control subjects (OR = 0.19, 95% CI = 0.03-1.38). These findings suggest that the +331G/A progesterone receptor promoter polymorphism may modify the molecular epidemiologic pathway that encompasses both the development of endometriosis and its subsequent transformation into endometrioid or clear cell ovarian cancer.



We found that there was a trend for increased ovarian cancer risk associated with the L allele of the **SRD5A2** V89L polymorphism (age adj OR=1.30; 95% CI=1.03-1.62;  $p_{\text{trend}}=0.03$ ) which was also apparent among the invasive tumours only (OR=1.25; 95% CI=0.98-1.59;  $p_{\text{trend}}=0.08$ ). A significant trend was observed for reduced ovarian cancer risk associated with the R allele of the **HSD17B4** W511R polymorphism (age adj OR=0.68; 95% CI=0.47-0.97;  $p_{\text{trend}}=0.04$ ); the trend remained similar for the invasive tumours (age adj OR=0.75; 95% CI=0.51-1.09;  $p_{\text{trend}}=0.14$ ). Genotype frequency differences across ovarian cancer subgroups defined by tumour characteristics (including histology) are being explored.

**Task 3 To genotype the cases and controls from the Australian Ovarian Cancer Study for 16 SNPs (months 25-42)**

Genotyping of the AOCS cases and controls has not yet started because collection is not yet finished. However, we have extracted DNAs from 775 AOCS cases and controls, and have also obtained access to 246 appropriate control DNAs from a related project, the Australian Cancer Study (PI – Professor Adele Green). These DNAs are currently being quantitated and plated into 384-well plates in preparation for genotyping. We have also changed our SNP genotyping platform and now use the Sequenom Mass Array system will allow much higher throughput, and higher levels of multiplexing. We therefore anticipate that all the required genotyping will be done within a short time of the completion of blood collection.

**Task 4 To perform genotyping for 2 short tandem repeat (STR) polymorphisms on both case-control studies (months 36-42)**

Not started because blood collection is ongoing (see above).

**Task 5 Statistical analysis of the genotyping results from the Survey of Women's Health Study and controls from the Australian Breast Cancer Family Study (months 24-36)**

Not started because genotyping has not finished (see above).

**Task 6 Full statistical analysis of the genotyping results (months 40-48) and preparation of manuscripts**

---

## PUBLICATIONS

Auranen, A., Spurdle, A.B., Chen, X., Lipscombe, J., Purdie, D.M., Hopper, J.L., Green, A., Healey, C.S., Redman, K., Dunning, A. M., Pharoah, P. D., Easton, D., Ponder, B.A.J., Chenevix-Trench, G., and Novik, K.L. BRCA2 Asn372His polymorphism and epithelial ovarian cancer risk. *International Journal of Cancer* 103:427-30 (2003)

Dunning AM, Dowsett M, Healey CS, Tee L, Luben RN, Folkard E, Novik KL, Kelemen L, Ogata S, Pharoah PD, Easton DF, Day NE, Ponder BA. Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J Natl Cancer Inst.* 96(12):936-45 (2004)



Berchuck, A., Shildkraut, J., Wenham, R.M., Calingaert, B., Ali, S., Henriott, A., Halabi, S., Rodriguez, G.C., Gertig, D., Purdie, D.M., Keleman, L., Spurdle, A.B., Marks, J. and **Chenevix-Trench, G.** Progesterone receptor +331A polymorphism is associated with a reduced risk of endometrioid and clear cell ovarian cancers. *Cancer Epidemiology, Biomarkers and Prevention* (in press)

Webb, P.M., Hopper, J.L., Newman, B., Chen, X., Keleman, L., McCredie, M.R.E., Southey, M., Giles, G.G., **Chenevix-Trench, G.** and Spurdle, A.B. Double strand break repair gene polymorphisms and risk of breast or ovarian cancer. *Cancer Epidemiology, Biomarkers and Prevention* (in press)

Keleman, L., Spurdle, A.B., Purdie, D.M., Gertig, D. and **Chenevix-Trench, G.** *RAD52* Y415X truncation polymorphism and epithelial ovarian cancer risk in Australian women. *Cancer Letters* (provisional acceptance)

## KEY RESEARCH ACCOMPLISHMENTS

### Cores A and B

We have established a network of research nurses across the country and recruitment is now progressing well at 15 different sites (See Reports for Core A Epidemiology and Core B Biospecimens). In relation to this we also have established systems to manage data and samples from all of the different sites.

### Project 1

Gene expression markers of potential prognostic significance have been identified and these need to be validated on additional datasets. We have identified a set of genes with robust expression differences between LMP and invasive tumours. We have also developed a classification tool for Krukenberg and Krukenberg-like tumours, which is likely to be of considerable value for determining the origin of atypical ovarian tumours, especially mucinous ovarian tumours.

### Project 2

This project will not formally commence until epidemiologic data collection is complete and it will run through the 4<sup>th</sup> year of the program.

### Project 3

The most recent novel finding from the samples from the Survey of Women's Health, in collaboration with Dr Andrew Berchuck, is that the +331A allele of the PR gene is significantly associated with protection against endometrioid ovarian cancers (OR 0.46, 95% CI = 0.23–0.92).

## REPORTABLE OUTCOMES

N/A

## CONCLUSIONS

### Cores A and B

Recruitment of cases for the study began in January 2003 and control recruitment began in May 2003. We have since recruited a total of 693 women with ovarian cancer and 437 control women. The recruitment, sample and data collection and processing systems are working well

and we are continuously monitoring our performance against our targets as outlined in the reports from Core Components A (Epidemiology) and B (Biospecimens).

### **Project 1**

Initial array analysis on archival samples has been in progress throughout 2004 leading to the discovery of several marker genes. We have now switched further microarray expression analysis of ovarian cancer samples to an Affymetrix U1332.0+ array system. The intention is to array 500 samples, including the previously tested archival samples and the, as yet untested, prospective samples on U1332.0+ arrays, thereby creating a database of 100's of well-defined ovarian samples.

### **Project 2**

This project will not formally commence until epidemiologic data collection is complete and it will run through the 4<sup>th</sup> year of the program.

### **Project 3**

Of the eleven polymorphisms nominated for analysis in this project, there is preliminary evidence from the SWH that four of them (in *PR*, *BRCA2*, *SRD5A2* and *HSD17B4*) are associated with ovarian cancer risk. Further analyses in the AOCS samples will provide independent testing of these SNPs in ovarian cancer risk, and if confirmed provide more power to look for associations with subtypes of ovarian cancer, and to start to look for gene-gene and gene-environment interactions

### **REFERENCES**

N/A

### **APPENDICES**

N/A